MICROFLUIDIC PLATFORMS FOR USE WITH SPECIFIC BINDING ASSAYS, SPECIFIC BINDING ASSAYS THAT EMPLOY MICROFLUIDICS, AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] Under the provisions of 35 U.S.C. §119(e), priority is claimed from U.S. Provisional Application Serial No. 60/351,261, filed on Jan. 23, 2002.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates generally to specific binding assays and, more specifically, to specific binding assay formats for analyzing very small samples or sample solutions. In particular, the present invention relates to specific binding assays that employ microfluidics to convey small samples or sample solutions across a number of different sensing zones and to microfluidic platforms for use with specific binding assays, as well as to methods for fabricating such microfluidic platforms and specific binding apparatus that include such microfluidic platforms.

[0004] 2. Background of Related Art

[0005] A major challenge in many biosensing applications is the real-time detection of a multitude of analytes from a small sample volume. Biological sensing has been an intensely active area of research due to applications in environmental sensing, food testing, and clinical screenings, just to name a few, and may include, for example, assays for cells, viruses, antibodies, proteins or peptides, nucleic acids, drugs, and other molecules of interest.

[0006] Many optical techniques have been studied for biosensing applications in which the analyte binds specifically (through an affinity interaction) to a capture molecule immobilized to the surface of a waveguide, and have proven to have relatively high sensitivity and to provide short assay times. These biosensors can be classified into two categories: mass sensors and fluorescence sensors. Mass sensors measure the presence of the captured analyte by detecting changes in absorption or refractive index, but are ineffective for analytes with small molecular weights and are sensitive to both specific binding and non-specific binding. Fluorescence sensors measure the emission from an immobilized tracer molecule or fluorescently-labeled analyte, which is excited by the evanescent field of an optical waveguide, and are generally more sensitive and more specific than mass sensors. Many of the fluorescence approaches are based on the use of evanescent wave excitation from an optical fiber or planar waveguide. Planar wave guides have many advantages over fibers, including larger sensing area, direct extension to multi-analyte array sensing, and support of integrated fluidic channels or flow cells.

[0007] Many specific binding, or affinity, biosensing techniques are based on introduction of a sample solution onto a chip or device, where the solution covers substantially the entire device (i.e., all of the sensing zones) and remains stationary during the sensing process. This process is highly inefficient in terms of the use of the sample volume and is the primary reason why molecular amplification steps are often taken in order to increase the sample volume. Molecular amplification, however, takes time and presents an additional step whereby the probability for introducing error into the test is increased. In addition, many existing assay techniques employ a so-called "end point" detection, which requires that the affinity reaction reach completion and, thus, further waiting.

[0008] Various approaches have been taken to facilitate the analysis of samples having small volumes. For example, U.S. Pat. No. 5,583,281, issued to Yu on Dec. 10, 1996 (hereinafter "Yu"), and U.S. Pat. No. 4,471,647, issued to Jerman et al. on Sep. 18, 1984 (hereinafter "Jerman"), disclose miniature gas chromatographs that include columns with spiral paths. As is typical with gas chromatographs, the constituent parts of a sample become separated from one another as the sample travels along the length of the column rather than by interaction with one or more reagents at sensing zones located along the length of the column.

[0009] Microfluidics have also been used in the analysis of liquid samples. Examples of the this use are provided by U.S. Pat. No. 5,641,400, issued to Kaltenbach et al. on Jun. 24, 1997 (hereinafter "Kaltenbach"), and U.S. Pat. No. 5,571,410, issued to Swedberg et al. on Nov. 5, 1996 (hereinafter "Swedberg"). Kaltenbach and Swedberg both disclose liquid phase sample separation apparatus that include laser-ablated microchannels that take somewhat serpentine paths. These apparatus may be used in electrophoretic separation processes and analytes that have been separated along the lengths of their microchannels may be detected by way of known optical processes (e.g., by measuring the absorbance at one or more particular wavelengths). Neither of these devices would, however, be useful in a real-time, optical, specific binding assay.

[0010] U.S. Pat. No. 5,482,598, issued to Isaka et al. on Jan. 9, 1996 (hereinafter "Isaka"), discloses a sample separation apparatus that includes a microchannel formed from porous silicon. This microchannel may have a somewhat spiral path. Again, however, the separation of one or more analytes from a sample is based on the size of each analyte, and the detection of each analyte is not effected until that analyte or a modified form thereof exits the microchannel.

[0011] Considerable work involving microfluidics and DNA is being performed. Dobrinski, H, et al., "Flexible Microfluidic-Device-Stamp-System with Integrated Electrical Sensor for Real Time DNA Detection," 1st Ann. Intern'l IEEE-EMBS Special Topic Conf. on Microtech. in Med. & Biol., pages 33-35 (Oct. 12-14, 2000), describes a DNA sensor that incorporates a silicon-polymer hybrid microfluidic flow cell. That flow cell is configured to spread a sample out over a single reaction area. Capture oligonucleotides within the reaction area are bound to a surface of the flow cell and a sample that includes DNA is flowed over the surface of the flow cell and past the capture oligonucleotides thereon to promote hybridization of the DNA therein with the immobilized capture oligonucleotides. Although detection is performed in real time, impedimetric techniques, rather than optical sensing processes, are employed.

[0012] The usefulness of microfluidics with end-point sensors is also being researched. For example, Kuhr et al. have developed an end-point sensor with which DNA may be electrochemically detected. Once analyte DNA has hybridized with capture oligonucleotides and the remainder of a sample solution has been flowed or washed away, the